

Protein Synthesis Lab

Purpose:

Recognize that the sequence of bases in DNA determines the shape, structure, and function of a protein.

Materials:

- Scissors
- White paper
- Pencil
- Construction paper
- Lab handout
- Tape

Background:

DNA is the molecule that stores the genetic information in your cells. That information is coded in the four bases of DNA: C, G, A, and T. The DNA directs the functions of the cell on a daily basis and will also be used to pass on the genetic information to the next generation. Because of its critical role in all the functions of the cell, DNA is kept protected in the nucleus of eukaryotic cells.

DNA is organized into sections called genes. Gene code for proteins, and it is proteins that do all the work in the cell. They function as structural proteins that serve as the building blocks of cells and bodies. They also function as enzymes, who are the little molecular chemists in bodies.

Proteins are made in the cytoplasm by protein factories called ribosomes. So, the information from DNA must be transmitted from the nucleus to the cytoplasm. Each gene on the DNA is read and codes directly for mRNA molecules. The mRNA is made by matching its complementary bases- C, G, A, and U to the DNA bases. The mRNA molecule then leaves the nucleus and carries the code for making the p[rotein from the DNA gene to the ribosome in the cytoplasm.

The ribosome reads the sequence of bases on the mRNA in sets of three called codons. Another type of RNA, tRNA, brings the protein building blocks, amino acids, to the ribosome as they are needed. The ribosome bonds the amino acids together to build the protein coded for by the gene back in the nucleus.

Procedure:

1. Your group should obtain one sheet of paper with your four sections of DNA. Cut the strips out along straight lines and tape them together to make a long one-sided DNA molecule. Each section is numbered. Lay them out on the desk from left (#1) to right (#4). See the diagram below.



2. We are going to use this section of our DNA as a gene to make a protein the cell needs. Remember it used to be part of a double-stranded DNA molecule. But it has already been unzipped and now will be used as the template to build your mRNA, one base at a time. So first design an RNA polymerase enzyme to do this mRNA synthesis job.
3. **TRANSCRIPTION:** You have been supplied with mRNA nucleotide bases. Build a mRNA molecule from this gene by matching the mRNA bases to your DNA template, one base at a time. Tape this mRNA molecule along its length to simulate the strong bonds that the RNA polymerase makes between the mRNA bases. This way, it will be a stable molecule and can

Modeling Protein Synthesis Lab Answers

John Patrick Welsh



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Chapter Resource 10 How Proteins/Made Biology Holt Rinehart & Winston, Holt, Rinehart and Winston Staff, 2004

Cell and Molecular Biology and Lab Work Mr. Rohit Manglik, 2024-06-24 A combined theoretical and practical approach to the study of cell and molecular biology with detailed lab exercises and methods *Creating Life in the Lab* Fazale Rana, 2011-02-01 Each year brings to light new scientific discoveries that have the power to either test our faith or strengthen it most recently the news that scientists have created artificial life forms in the laboratory If humans can create life what does that mean for the creation story found in Scripture Biochemist and Christian apologist Fazale Rana for one isn't worried In *Creating Life in the Lab* he details the fascinating quest for synthetic life and argues convincingly that when scientists succeed in creating life in the lab they will unwittingly undermine the evolutionary explanation for the origin of life demonstrating instead that undirected chemical processes cannot produce a living entity Biology Eric Strauss, Marilyn Lisowski, 2000 **Production of Complex Heterologous Proteins and Protein Assemblies Using E. Coli-based**

Cell-free Protein Synthesis John Patrick Welsh, 2011 The Swartz lab has put much effort into understanding the underlying principles of E coli based cell free protein synthesis CFPS and the technology has developed into a scalable affordable platform for producing a wide range of protein targets Key breakthroughs have included activating central metabolism stabilization of critical amino acids controlling the redox environment to produce proteins containing disulfide bonds and using scale up technologies to produce proteins at milligram quantities My work has sought to expand this CFPS technology for producing valuable and complex eukaryotic protein targets by manipulating and optimizing the folding of these proteins in the heterologous CFPS environment Furthermore I have sought to apply these advances to specific applications of interest By modifying a key molecular chaperone native to the eukaryotic endoplasmic reticulum ER the Hsp70 family chaperone BiP soluble production was increased in CFPS reactions for specific proteins normally secreted through this organelle namely those from the immunoglobulin superfamily which includes antibodies T cell receptors and many membrane receptors First the functional properties of BiP were compared to that of the E coli Hsp70 DnaK A fusion protein was then constructed between BiP and the ribosome binding portion of the E coli protein trigger factor to localize BiP to translating ribosomes This replicated the native function of BiP which provides co translational folding assistance to nascent polypeptides After verifying its bioactivity this fusion protein was utilized in CFPS reactions to indicate that the chaperone functions of BiP are specific to proteins normally secreted through the eukaryotic ER whereas DnaK demonstrates a more general chaperone mechanism Since the discovery that somatic cells could be reprogrammed back to a pluripotent state through the viral expression of a specific set of transcription factors there has been great interest in reprogramming using a safer and more clinically relevant protein based approach Production of these transcription factor proteins was greatly increased by fusing them to the C terminus of the solubility partner IF2 domain 1 IF2D1 While the fusions provided marginal benefit in molar yields using a

CFPS approach in vivo E coli expression produced the transcription factors in soluble form The fusion proteins could be purified in milligram quantities from liter shake flask cultures whereas essentially no soluble protein accumulated without the fusion partner The transcription factor fusions bound specifically to their consensus DNA sequences and partially activated some of their downstream gene targets Another application utilizing CFPS technology is an enhanced luciferase mutant from the marine copepod *Gaussia princeps* GLuc GLuc is both the smallest and brightest known luciferase and previous work from our lab demonstrated that this protein could be produced at higher volumetric yields and specific activities in CFPS compared to conventional protein expression systems By mutating key residues in the *Gaussia* luciferase sequence the luminescence half life was shown to increase over ten fold while maintaining the initial specific activity of the wild type This improved mutant provides a valuable imaging agent to use in fusions and bioconjugates with other proteins such as those that recognize cell surface markers on cancer cells In a final application influenza vaccines were produced using CFPS by isolating specific fragments of the protein hemagglutinin HA a viral surface protein Specific mutations as well as a C terminal trimerization domain were critical for producing this protein fragment in both its monomeric and native trimeric forms By using the CFPS platform to incorporate non natural amino acids nnAAs with alkyne functional groups the HA proteins were covalently clicked to virus like particles VLPs that had surface exposed nnAAs with azide functionality The VLPs provide an immunogenic delivery platform that efficiently traffics to lymph nodes and allows for co attachment of other adjuvants in addition to the primary HA antigen This vaccine platform was characterized and tested in mouse models and compared to both a standard influenza vaccine as well as free HA protein fragments In summary CFPS is a valuable and robust method of protein production for a variety of targets My thesis has sought to use this platform as a means to better understand folding pathways of complex eukaryotic proteins and improve production of these proteins To this end CFPS has been shown to be a valuable method for elucidating and manipulating chaperone function producing difficult proteins with enhanced function and as a platform to produce novel vaccines

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